UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service

ARS 52-54

Effect of Methods of Preservation and Storage of Fecal Samples on Energy Losses[1/]

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The importance of extreme accuracy in all analytical methods used in calorimetric studies involving balance techniques has been stressed repeatedly, and some of the laboratories engaged in this type of research have reported the attainment of phenomenally high orders of analytical precision. However, if there have been unaccountable losses of energy during the preservation, storage and preparation of the sample for analysis much of the advantage gained by analytical accuracy is lost. The usual procedure for determining the caloric value of feces has been to burn a dry sample in a bomb calorimeter, and most of the energy values reported in the literature have been based on this method.

The recent work of Colovos *et al.* (1957) illustrated the possible magnitude of energy losses which may occur during the drying of fecal samples. Their results showed losses of energy ranging from 4.1 to 21.0% (ave. 13.8%) when bovine feces were dried at 65°C. Some of the early German workers observed a loss of carbon and nitrogen during the drying of feces, and various precautions were made to reduce the losses or to correct them. One of the methods used was to determine carbon and nitrogen on the fresh material as well as on the dried sample and calculate corrections for the heat of combustion determined on the dry material. Another technique was to dry the fecal samples in an apparatus with a combustion and absorption train to collect volatile gases and CO_2 lost during drying. Other methods of reducing losses have been by the use of preservatives, frequent sampling, drying under high vacuum at room temperature, and drying rapidly at 100° C. The method described by Colovos *et al.* (1957) of adding a primer to moist feces and determining the caloric value of the fresh material appears to be the simplest and most practical solution to the problem of losses occurring during the preservation, storage and drying of fecal samples.

1/ Paper presented at the annual meeting of the American Dairy Science Association, June 26-29, 1957 at Oklahoma A&M College, Stillwater, Oklahoma.

The present study was conducted to obtain quantitative data on the extent of energy losses resulting from preserving and drying bovine feces. Samples of feces were collected daily from three dry Jersey cows which were on a conventional total collection digestion trial involving a 3 x 3 Latin square design. The rations consisted of three lots of orchard grass hay, and each collection period was for 5 consecutive days. Urine and feces were separated by means of a rotating belt device.

Samples were taken from each day's fecal collection of each cow and treated in the following ways: I. Fresh-analyzed immediately; II. Dried in a hot air drier at 80°C; III. Canned and autoclaved. The feces were canned immediately by the method described by Jacobson et al. (1957) and were stored at room temperature until opened for analysis. Gross energy determinations were made with an oxygen bomb calorimeter using a modification of the technique of Colovos et al. (1957). Combustion of moist samples (1.5 to 2.5 g) including the fresh and canned feces, was facilitated by the addition of 2 ml. of 95% ethanol as a primer. The alcohol was maintained at 44-46°F to reduce evaporative losses during addition and mixing with the sample. Pyrex glass rods 3 cm x 2 mm. were used to mix the feces and alcohol immediately before the bomb was sealed. The gross energy of the alcohol (5,235 cal./ml.) was subtracted from the total gross energy value to obtain the caloric value of the feces. All values were calculated on the oven dry matter basis for purposes of comparison.

Results and Discussion

Table 1 summarizes the results obtained. The values for period I have been omitted because of incomplete combustion of several of the fresh samples due to failure to add enough alcohol and insufficient mixing. No difficulties in combustion of moist samples were encountered after the first week. The loss (3.27 ± 3.08%, standard deviation) due to drying in a hot air drier maintained at 80°C was highly significant (P = 0.01), the caloric value being 5063 ± 133 cal./gm. dry matter (DM) for fresh feces and 4893 ± 29 cal./gm. DM for dried feces. Canning and autoclaving feces prevented energy losses due to storage, the caloric value being 5090 ± 96 cal./gm. DM. A comparison of the per cent dry matter of the canned and fresh feces indicated that there was no change in dry matter due to canning. The average per cent dry matter of the fresh material was 14.82, while the dry matter content of canned feces was 14.83%.

Statistical analysis of the losses due to drying revealed that there were no significant differences due to cows, periods x cows, or days within periods, but there was a highly significant difference (P = 0.01) between periods. One explanation for this difference could be differences in the efficiency of the drier during different periods because of the presence of varying amounts of other moist materials being dried. Another reason could have been analytical or sampling errors. Sampling and analytical errors were much greater for the moist determinations than for the dried,

with the standard error of duplicate analyses being 1.86% for fresh, 1.34% for canned, and 0.42% for dried samples. The alcohol added to the moist sample contributed almost 85% of the gross calories, so an extremely small error in the amount or gross energy value of the alcohol added resulted in a relatively large error in the caloric value of the feces. Further refinements of the alcohol priming technique, such as the use of a precision syringe pipette, could possibly reduce the analytical errors, but sampling errors would remain. Another factor in the use of the alcohol priming technique which should be considered is the manner of determining the gross energy of the alcohol. The average of five determinations in which alcohol alone was burned was 5,235 cal./ml. When the alcohol walue was determined by adding 2 ml. of alcohol to a dry sample of feces of known caloric value plus 2 ml. of distilled water to make the moisture content approximately the same as the fresh feces, the average of six determinations was 5,215 cal./ml.

The relatively small losses of energy due to drying (3.27%) as compared to the results obtained by Colovos et al.(13.8% may be partially explained by the differences in the temperature at which the feces were dried. Colovos et al.(1957) dried their samples in a convection oven for 48 hours at about 65°C. The samples used in this study were dried to a constant weight (2-4 days) in a hot air drier maintained at 80°C, and the results are substantially in agreement with the findings of Kane et al. (1953) in that there was no loss of dry matter of fecal samples on drying at 80°C. According to Raymond and Harris (1954) respiration in herbage samples continues until the sample is dehydrated or heated to a temperature high enough to inactivate the enzyme systems and/or bacterial action, and the same explanation may be applicable for fecal energy losses. Other factors which may have contributed to the differences could have been the extent of urinary contamination of the feces, dietary differences, and the gross energy value of the alcohol used for priming.

Summary

A comparison of the caloric values of bovine feces analyzed immediately, dried at 80°C, and canned indicated that there was a 3.27± 3.08% loss of energy due to drying, and that canning feces prevented energy losses due to storage. The use of alcohol as a primer to facilitate combustion of moist samples was simple and rapid, but the precision was less than that obtained with dry samples.

References Cited

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Table 1

Dry matter and gross energy values of bovine feces prepared and stored by different methods.*

Cow No.	Period and Day No.	Dry Matter vacuum oven 100°C Fresh	Dry Matter vacuum oven 100°C Canned	Calories/gram Dry Matter Fresh	Calories/gram Dry Matter Dried	Calories/gram Dry Matter Canned	% Recovery Comparisons Dried/Fresh	% Recovery Comparisons Dried/Canned	% Recovery Comparisons Canned/Fresh
348	II - 1	12.51	12.66	5183	4822	5049	93.02	95.50	97.41
	II - 2	14.27	14.19	5260	4940	5103	93.92	96.82	97.00
	II - 3	13.94	13.93	5255	4940	4901	94.00	100.30	93.26
	II - 4	14.20	14.29	5152	4923	4778	95.56	103.04	93.26
	II - 5	15.62	15.67	5098	4904	5096	96.19	96.24	99.96
681	II - 1	14.78	14.70	5210	4905	4992	94.15	98.25	95.83
	II - 2	15.06	15.04	5179	4824	5193	93.16	92.90	100.28
	II - 3	14.74	14.72	5001	4865	4946	97.28	98.38	98.88
	II - 4	14.64	14.73	5064	4834	5048	95.45	95.75	99.69
	II - 5	14.89	15.05	5121	4845	5047	94.62	96.00	98.56
682	II - 1	12.83	12.85	5126	4915	5246	95.89	93.70	102.34
	II - 2	14.29	14.28	5236	4876	5116	93.12	95.31	97.70
	II - 3	14.45	14.41	4778	4890	4822	102.34	101.39	100.94
	II - 4	13.96	14.04	5059	4819	4984	95.25	96.69	98.51
	II - 5	14.02	14.04	5088	4869	5010	95.69	97.18	98.46
348	III - 1	16.93	16.99	5411	4972	5234	91.87	94.99	96.72
	III - 2	15.65	15.67	5001	4961	5204	99.20	95.33	104.06
	III - 3	14.65	14.58	5032	4910	5058	97.58	97.07	100.52
	III - 4	14.77	14.69	4795	4921	5096	102.62	96.56	106.28
	III - 5	14.93	14.84	5068	4858	5122	95.86	94.84	101.08
681	III - 1	15.37	13.38	5286	4992	5062	94.43	98.62	95.75
	III - 2	15.55	15.61	5058	4817	5236	95.25	92.00	103.54
	III - 3	15.80	15.87	4917	4885	5265	99.36	92.79	107.09
	III - 4	15.72	15.49	4751	4910	5182	103.35	94.76	109.06
	III - 5	15.81	15.83	5023	4900	5146	97.55	95.23	102.44
682	III - 1	15.04	15.06	5062	4920	5085	97.18	96.76	100.44
	III - 2	14.99	15.13	5047	4922	5129	97.52	95.97	101.61
	III - 3	15.08	15.16	4774	4872	5317	102.06	91.64	111.37
	III - 4	15.62	15.40	4837	4872	5317	100.73	94.49	106.61
	III - 5	14.58	14.65	5009	4894	5066	97.70	96.61	101.13
	Average	14.82	14.83	5063	4893	5090	96.73	96.19	100.66
	Standard deviation			133	27	96	3.08	2.57	4.33
	Standard error of duplicates			94	21	68			

* Each value is the average of two analyses.